REMARKS

The status of the application is as follows:

Original Claims 1-12 were presented for prosecution.

Original Claims 7 and 12 were withdrawn from consideration by the Examiner as being drawn to non-elected subject matter, and were cancelled by the Applicant.

Original Claims 1-6 and 8-11, as previously amended, were rejected by the Examiner.

Original Claims 1-6 and 8-11 were previously amended and are further amended or cancelled herein.

No Claims have been allowed.

Original Claims 1-6 and 8-11 presently remain pending for reconsideration by the Examiner.

The Examiner objected to Claims 1, 3, 6, 8, 9 and 11 under 35 U.S.C. § 112 paragraph 2 for lack of clarity with respect to certain terms, requesting clarification via clearer claim language.

The Examiner rejected Claims 1 and 2 under 35 U.S.C. § 103(a), as being allegedly unpatentable over:

1) Doi, N. & Yanagawa, H., *Minireview Insertional Gene Fusion Technology*, FEBS Letters 457 (1999) 1-4 (**Doi et al**), in view of;
2) Sevcik, J, et al., *X-ray Structure of Two Crystalline Forms of Streptomycete*

Ribonuclease with Cytotoxic Activity, Journal of Biological Chemistry, Vol. 277, No. 49, Issue of December 6, pp. 47325-47330 (**Sevcik et al**);

3) Hochstrasser, M., *Evolution and Function of Ubiquitin-like Protein-conjugation Systems*, Nature Cell Biology, Vol. 2, Aug. 2000 (**Hochstrasser**);
4) Varshavsky, A., *Codominant Interference, Antieffectors, and Multitarget Drugs*, Proc. Natl. Acad. Sci. USA 95 pp. 2094-2099 (**Varshavsky**);
5) Wintrode, P., et al., *Thermodynamics of Ubiquitin Unfolding*, PROTEINS: Structure, Function and Genetics 18:246-253 (1994) (**Wintrode et al**);
6) Pace, N., et al., *Urea Denaturation of Barnase: pH Dependence and Characterization of the Unfolded State*, Biochemistry, Vol. 21, No. 19, 1992 (**Pace et al**);

[DETAILED ACTION: NOTICE OF REFERENCES CITED]

Applicants respectfully reply to the Examiner's 35 U.S.C. § 112 paragraph 2 and 35 U.S.C. § 103(a) rejections hereinbelow, and request reconsideration in light of the foregoing amendments and accompanying remarks that follow. In view of the amendments herein, applicants respectfully submit that all of the pending claims are allowable over the prior art of record.

REMARKS RESPONSIVE TO EXAMINER'S REQUEST FOR CLARIFICATION OF CERTAIN CLAIM LANGUAGE UNDER 35 U.S.C. § 112 PARAGRAPH 2

The Examiner objected to Claims 1, 3, 6, 8, 9 and 11 for lack of clarity with respect to certain terms used therein, requesting clarification via clearer claim language.

In response, Claims 1, 3 and 11 have been amended; and, Claims 6, 8 and 9 have been cancelled. Applicants request reconsideration in light of the foregoing cancellations, clarifying amendments and accompanying remarks that follow. In view of the amendments herein, applicants respectfully submit that all of the pending claims are allowable over the prior art of record.

The Examiner Stated that the limitation

> "the domain being associated with a ... quantity of free energy ..."

in Claim 1 appeared to be a method step; however, as the claimed invention is directed to a fusion protein, it was unclear to the Examiner whether this limitation was an intended use of the free energy, an active method step, or a limitation of the fusion protein. If the latter, it was unclear to the Examiner in what way this limitation further limits the claimed fusion protein. Clarification was requested via clearer claim language.

[DETAILED ACTION: PAGE 3; LAST FULL PARAGRAPH]

In response, Applicants have amended Claim 1 to clarify to delete this "ambiguous" limitation.

The Examiner stated that the limitation

> "wherein all or part of the... quantity of free energy is made available to drive a folding of the target... domain..."

in Claim 3 appeared to be a method step; however, as the claimed invention is directed to a fusion protein, it was unclear to the Examiner whether this limitation was an intended use of said free energy, an active method step, or a limitation of the fusion protein. If the latter, it was unclear to the Examiner in what way this above limitation further limits the claimed fusion protein. Clarification was requested via clearer claim language.

[DETAILED ACTION: PAGE 4; FIRST FULL PARAGRAPH]

In response, Applicants have amended Claim 3 to clarify that the claimed fusion protein has the property that its regulatory domain or cytotoxic domains may fold under the influence of an effector signal.

The Examiner stated that the limitation

> "wherein the insert domain and the target domain are disenabled from

simultaneously coexisting",

in Claim 6 appeared to be a method step; however, as the claimed invention is directed to a fusion protein, it was unclear to the Examiner whether this limitation was intended to be an active method step or a limitation of the fusion protein.
[DETAILED ACTION: PAGE 4; SECOND FULL PARAGRAPH]

In response, Applicants have cancelled Claim 6.

The Examiner stated that the limitation

"wherein any excess of the first quantity of free energy... is spontaneously transferred",

in Claim 8 appeared to be a method step; however, as the claimed invention is directed to a fusion protein, it was unclear to the Examiner in what way this limitation further limits the claimed fusion protein.
[DETAILED ACTION: PAGE 4; THIRD FULL PARAGRAPH]

In response, Applicants have cancelled Claim 8.

The Examiner stated that the limitation

"is spontaneously transferred",

in Claim 9 appeared to be a method step; however, as the claimed invention is directed to a fusion protein, it was unclear to the Examiner in what way this limitation further limits the claimed fusion protein.
[DETAILED ACTION: PAGE 4; FOURTH FULL PARAGRAPH]

In response, Applicants have cancelled Claim 9.

The Examiner stated that the limitation

> "that may be determined by the ... effector signals",

in Claim 11 appears to be a method step; however, as the claimed invention is directed to a fusion protein, it was unclear to the Examiner whether this limitation is an intended use, an active method step, or a limitation of the fusion protein. If the latter, it was unclear to the Examiner in what way the above limitation further limits the claimed fusion protein; and, clarification was requested via clearer claim language.
[DETAILED ACTION: PAGE 4; LAST PARAPGRAPH]

In response, Applicants have amended Claim 11 to clarify that the cooperative and reversible conformational equilibrium exhibited by the claimed fusion protein has the property that it may be influenced by an effector signal.

REMARKS RESPONSIVE TO 35 U.S.C. § 103(a) REJECTION

The Examiner rejected Claims 1 and 2 under 35 U.S.C. 103(a) as being made obvious by the Primary Reference **Doi et al**, in view of the Secondary References: **Sevcik et al**; **Hochstrasser**; and, **Varshavsky.**

The Examiner's 1st Argument in Reliance upon Doi et al
The Examiner asserted that **Doi et al** teach methods for engineering insertional fusion proteins [Abstract] and [Table 1];
[DETAILED ACTION: PAGE 5; LAST FULL PARAGRAPH]

Applicants initially point out to the Examiner that neither Claim 1 nor Claim 2 is directed to a ***method***. Rather, Claims 1 and Claim 2 claim a ubiquitin-barnase ***fusion protein***, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein." This invention is not taught, suggested or practiced by **Doi et al.**

Moreover, to the extent that the Examiner contends that "it would have been obvious to someone of ordinary skill in the art at the time of the instant invention to create a mutually exclusive protein using the ***method*** of Doi et al ... ." [DETAILED ACTION: PAGE 7; FIRST FULL PARAGRAPH] , a reading of **Doi et** al reveals that they do not teach any method for creating a fusion

protein, other than in principle.

The Examiner's Citation of The Abstract within Doi et al

The Abstract in **Doi et al**, cited by the Examiner, states, *inter alia*, that:

> "[i]n this review, we ... compare the old and new gene fusion techniques".

This statement in **Doi et al** refers to "techniques" but teaches or suggests teaches nothing about a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein" as in Claims 1 and 2.

The Examiner's Citation of TABLE I within Doi et al

Table 1 in **Doi et al**, cited by the Examiner, is simply a compilation of fusion proteins, none of which teach or suggest a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein" as in Claims 1 and 2.

The Examiner's 2nd Argument in reliance upon Doi et al

The Examiner asserted that **Doi et al** teach the insertion of a sequence of proteins (i.e. insert protein) into a surface loop region of an enzyme (i.e. target domain) domains [Section 2.2], wherein insertions are between N-terminal and

C-terminals [Fig. 1 and 2], as in claim 1.
[DETAILED ACTION: PAGE 5; LAST FULL PARAGRAPH]

The Examiner's Citation of Section 2.2 within Doi et al
Section 2.2 of **Doi et al**, cited by the Examiner, is captioned

> "2.2. Display of randomized protein libraries",

And, this section states, in its only relevant phrase, that

> "random sequence proteins of more than 100 aa residues could be also inserted into a surface loop region of an enzyme, *Escherichia coli* RNAse HI."

However, this finding of what is ***possible*** with respect to the enzyme *Escherichia coli* RNAse HI, neither teaches nor suggests a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein" as in Claim 1.

The Examiner's Citation of Fig. 1 in Section 2.2 within Doi et al
Reference to Fig. 1 in Section 2.2 of **Doi et al**, also cited by the Examiner, is found in that Section's first sentence, which states that:

"The parent domain (Fig. 1) can serve as a scaffold to display random peptide or protein libraries."

Fig. 1 of **Doi et al** is, in turn, labeled:

"Schematic representation of the two types of gene fusion ***approaches***" [emphasis supplied],

and illustrates what is captioned in Fig. 1 as an "insertion" approach.

While Fig. 1 illustrates the schematic differences between the "end-to-end" fusion technique and the insertional fusion technique:

- Fig. 1 does not teach or suggests any methods for practicing either technique;
- Fig. 1 does not teaches or suggests a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein" as in Claim 1; and, Claim 1 is not directed to an "approach," i.e., a method.

The Examiner's 3rd Argument in reliance upon Doi et al

The Examiner asserted that **Doi et al** teach the design of biosensor proteins,

- wherein the activity if an insert domain is modulated by a conformational change of a target domain [Section 2.3, Col. 1] and

- wherein proteins with desired molecular recognition domains (i.e., regulatory domains are inserted into a target protein loop [Section 2.3, Col. 2, ¶2]

  as in claim 1.

[DETAILED ACTION: PAGE 5; LAST FULL PARAGRAPH TO PAGE 6; FIRST FULL PARAGRAPH]

The Examiner's Citation of Section 2.3, Column 1 within Doi et al

For the assertion that **Doi et al** teach the design of biosensor proteins, wherein the activity of an insert domain is modulated by a conformational change of a target domain, Section 2.3, Col. 1 of **Doi et al**, states, in relevant part that:

> "Betton et al. ... inserted TEM1-lactamase into the maltose binding protein MalE. ... [T]he penicillinase activity of the insert domain was modulated by a conformational change of MalE upon binding of maltose."

However, the fact that the penicillinase activity of TEM1 Beta-lactamase, a specific fusion protein, is influenced by a conformational change in MalE neither teaches nor suggests a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein" as in Claim 1.

Moreover, the unpredictability of the biological activity of any given protein

that is modified by as little as a change in a single amino acid is well established in the art. Consequently, the behavior of the TEM1 Beta-lactamase-MalE fusion protein, cited by the Examiner in **Doi et al** has no predictive or anticipatory relevance to Claim 1, other than to comport with the *fact* that proteins having regulatory domains ***may*** be inserted into a target protein loop. However, the fact that one protein may be insertionally fused with another protein neither teaches or suggests anything about a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein" as in Claim 1.

The Examiner's Citation of Section 2.3, Column 2, Paragraph 2 within Doi et al

For the assertion that **Doi et al** teach proteins with desired molecular recognition domains (i.e., regulatory domains are inserted into a target protein loop, Section 2.3, Col. 2, ¶2 of **Doi et al**, states, in relevant part that:

> "We have proposed a method of constructing generic GFP [green fluorescent protein]-based biosensors in which a desired molecular recognition domain is inserted into a loop of GFP... ."

Again, Claim 1 claims a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater

than an alpha-carbon-alpha-carbon length of the ... target protein." Nothing in the foregoing quotation or Section 2.3, Col. 2, ¶2 of **Doi et al**, in which the quotation is embedded, teaches or suggests the fusion protein claimed in Claim 1.

The Examiner's 4th Argument in reliance upon Doi et al

The Examiner asserted that **Doi et al** teach insert domains and parent domains wherein insert domains are over twice the length of parent domains [Fig. 1] and [Table 1], as in claim 1.
[DETAILED ACTION: PAGE 6; FIRST FULL PARAGRAPH]

The Examiner's Citation of Fig. 2 within Doi et al

Fig. 1 in **Doi et al**, cited by the Examiner, neither teaches nor suggests anything about the relative lengths of insert and parent (i.e., target) protein domains; and is consequently entirely inapposite.

The Examiner's Citation of Table 1 within Doi et al

Table 1 in **Doi et al**, cited by the Examiner for the assertion that **Doi et al** teach insert domains are over twice the length of parent domains, is captioned:

> "Engineered insertional fusion proteins",

and lists nine examples of insert domains paired with parent (i.e., target) domains. Adjacent any listed insert domain and a target domain, identified as components of an associated fusion protein, is a number enclosed in parentheses,

indicating the amino-acid length of the insert domain and target domain respectively

However, for none of the fusion proteins listed in Table 1, is it the case that the amino acid length of the insert domain is at least twice the amino acid length of the parent (target) domain. Indeed, in all of the examples appearing in Table 1 except two, the amino acid length of the insert domain is ***less than*** the amino acid of the parent domain. And, in the cases of *A. victoria* GFP (238)-Hepatitis B virus core protein (183) and TEM1 Beta-lactamase (263)-*A. victoria* GFP (238), the amino acid length of the insert protein is only ***slightly*** greater than the amino acid length of the parent (target) protein.

Consequently, Table 1 can, at best, only be cited for teaching that some fusion proteins were known **by Doi et al** as having insert domains whose ***amino acid lengths*** were ***slightly greater*** than the amino acid lengths of parent (target) domains. However, most of the fusion proteins listed in Table 1 **by Doi et al** have insert domains whose amino acid lengths are ***less than*** the amino acid lengths of parent (target) domains - - precisely the opposite of and teaching away from - - what is claimed in Claim 1, in which a ubiquitin-barnase fusion protein is claimed, as a composition of matter, the structure of which is limited by the explicit design constraint that “an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein”.

Moreover, even if, ***arguendo***, Table 1 teaches or suggests insert domains and

parent domains wherein insert domains are over twice the length of parent domains, as mistakenly asserted by the Examiner, that hypothetical teaching would apply exclusively to lengths measured by the number of amino acids in the insert domain and the number of amino acids in the parent (target) domain. However, the overall amino acid length of a protein domain teaches nothing about the dimensions of the domain as part of a fusion protein, because the amino acid chain may be folded in a variety of unpredictable conformations in a fusion protein. Amino acid lengths, standing alone, are simply not useful criteria for designing the structure of a fusion protein.

For this reason, the length limitation claimed in Claim is directed to

- an ***amino-carboxyl length*** of an insert protein (that is not measured by a count of amino acids); and,
- an ***alpha-carbon-alpha-carbon length*** (that is not measured by a count of amino acids) of a target protein.

The claimed amino-carboxyl length and alpha-carbon-alpha-carbon length are respectively and explicitly illustrated in Fig. 2A and Fig. 2B of the instant Application; and, are respectively defined in the instant Specification as follows:

- The amino terminal of the ... insert protein is spatially separated from the carboxyl terminal of the ... insert protein by a linear (i.e., straight line) distance known as the amino-carboxyl length ... that is measured when the ... insert protein is in its folded conformation~~.~~; see, e.g. double-headed arrow in FIG. 2A.
- The alpha carbon of the initial amino acid of the surface loop of the... target protein is spatially separated from the alpha carbon of the terminal amino

acid of the surface loop of the ... target protein by a linear (i.e., straight line) distance known as the alpha-carbon-alpha-carbon length of the surface loop of the ... target protein ... that is measured when the ...target protein is in its folded conformation ; see, e.g. double-headed arrow in FIG. 2B.

Significantly, these definitions make no reference to amino acid lengths whatever. Indeed, Applicants' Fig. 2A and Fig. 2B illustrate and specify lengths in angstrom units, not amino acids; and, the ambiguity of using an amino acid length as a structural design criterion for a fusion protein is made apparent in Applicants' Fig. 2A and Fig. 2B, wherein the folding of schematic amino acid chains stands in stark contrast the illustrated straight line dimensions upon which Claim 1 is predicated.

Summarizing from the foregoing, applicants respectfully contend that Claim 1 is not unpatentable over **Doi et** al standing alone because:

1) Table 1 of **Doi et al** does ***not*** teach or suggest insert domains that are over twice the length of parent domains; instead,
2) Table 1 of **Doi et al** teaches insert domains that ***less than*** the length of parent domains; and,
3) Claim 1 claims an insert domain that is at least twice the length of the parent (target) domains; and,
4) Claim 1 claims a ubiquitin-barnase fusion protein subject, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is

at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein"; and,

5) Claim 1 does not read on Table 1 of **Doi et al**.

The Examiner's 5th Argument in reliance upon Doi et al

The Examiner asserted that **Doi et al** teach insert proteins that switch between folded and unfolded conformations and are associated with high and low activity (i.e. energy) [Fig. 2], as in claim 2.
[DETAILED ACTION: PAGE 6; FIRST FULL PARAGRAPH]

Initially, Claim 2 is dependent upon claim 1, and accordingly all of the limitations of Claim 1 are read into Claim 2. As indicated above, the Examiner objected to the use of the limitation:

"the domain being associated with a ... quantity of free energy ..."

in Claim 1, which free energy the Examiner has now identified with the term "activity" appearing in **Doi et al.**

In response to the Examiner's objection, Applicants have amended Claim 1 herein to delete this limitation from Claim 1. Consequently, Claim 2 now has no reference to energy (or activity) whatsoever; and, the Examiner's assertion that **Doi et al** teach insert proteins that switch between folded and unfolded conformations and are associated with high and low activity (i.e. energy) has been rendered moot with respect to Claim 2.

The Examiner's Citation of Fig. 2 within Doi et al

Fig. 2 in **Doi et al** is captioned:

> "Applications of domain insertion for functional switching".

Applicants acknowledge that the fusion protein of Claim 2 has a ***use*** as a molecular switch, but make no claim for this use. Rather, Claim 2, depending from Claim 1, subjects the ubiquitin-barnase fusion protein of Claim 1 to the further limitation that "if the insert ... domain is in its folded conformation, the target ... domain is in its unfolded conformation and vice versa."

Accordingly, Claim 2 does not claim a ***use*** suggested by the caption of Fig. 2 of **Doi et al**; nor, does the caption of Fig. 2 teach "insert proteins that switch between folded and unfolded conformations and are associated with high and low activity (i.e. energy)".

The Examiner's Citation of Fig. 2A within Doi et al

Referring to Fig. 2A in Section 2.1, Column 2, ¶ 1, the text of **Doi et al** teach that alkaline phosphatase (AP) was inserted into various cytoplasmic and periplasmic loops of a multispanning membrane protein MalF, and:

> "exhibited a high ***activity*** when it was fused into a periplasmic loop of MalF, whereas the ***activity*** was very low when AP was inserted into a cytoplasmic loop of the membrane protein (Fig. 2A)." [emphasis supplied]

This quotation teaches that AP's ***enzymatic*** (i.e., biological) "activity", which the Examiner has mistakenly analogized to (free) "energy", is a function of its insertion locus in the parent (target) domain MalF. The quotation teaches away from Claim 2; and, certainly does ***not*** teach that if

> "the insert ... domain is in its folded conformation, the target ... domain is in its unfolded conformation and vice versa",

as in claim 2.

The Examiner's Citation of Fig. 2B within Doi et al

Referring next to Fig. 2B in Section 2.1, Column 2, ¶ 2, **Doi et al** teach that:

> "by monitoring the scaffold enzymatic activity, the hill-climbing of random sequence proteins on a foldability landscape can be analyzed."

Accordingly, **Doi et al** use the term "activity" to refer to "enzymatic activity" - - a measure of biological function and ***not*** a measure of free energy.

Additionally Fig. 2B of **Doi et al** teaches the analysis of the:
"enzymatic activity" of "random sequence proteins",
which teach away from, and consequently, are of no relevance to Claim 2.

Fig. 2B also contains the term "Directed evolution", which is nowhere defined by **Doi et al**, in this regard, **Doi et al**:

- at best, teach away from Claim 1; and,
- at worst, teach or suggest nothing about Claim 1; and certainly,
- does not teach or suggest "insert proteins that switch between folded and unfolded conformations and are associated with high and low activity (i.e. energy)", as asserted by the Examiner.

The Examiner's Citation of Fig. 2C within Doi et al

Referring next to Fig. 2C in Section 2.3, Column 2, last full ¶, **Doi et al** teach that:

> "[b]y using random mutagenesis of the insertional fusion protein and screening for BLIP [Beta-lactamase inhibitory protein] sensitivity, we obtained a sensor protein in which the GFP fluorescence increased upon binding of BLIP... ."

As Claim 2 makes no reference "random mutagenesis or "BLIP screening", **Doi et al** again teach away from Claim 2.

The Examiner's 6th Argument in reliance upon Doi et al

The Examiner acknowledged that **Doi et al** do not specifically teach fusion proteins comprising ubiquitin and barnase, as in claim 1. However, the Examiner asserted that **Doi et al**:

- teach the **use** of RNAse (ribonuclease) as a parent domain (i.e. target

protein), which suggests the use of proteins in the family of ribonucleases (e.g. barnase) [Fig. 2]; and,

- suggest this technique should be used for designing stable bifunctional proteins [P. 3, Col. 2, ¶1]

[DETAILED ACTION: PAGE 6; FIRST PARTIAL PARAGRAPH]

In referring to Fig. 2B in Section 2.1, Column 2, ¶ 2, **Doi et al** teach that:

> "[B]y monitoring the scaffold enzymatic activity, the hill-climbing of random sequence proteins on a foldability landscape can be analyzed. Not only RNAse HI, but also kanamycin nucleotidyltransferase and GFP can be used as scaffolds for displaying random sequence proteins ... ."

Fig. 2B of **Doi et al** illustrates the foregoing analysis of the "enzymatic activity" of "random sequence proteins". Clearly, **Doi et al** teach the use of RNAse HI "as scaffolds for displaying random sequence proteins", but this in no way suggests the use of proteins in the family of ribonucleases (e.g. barnase) for designing stable bifunctional proteins, as the Examiner contends.

Moreover, even if, *arguendo*, **Doi et al** suggest the use of RNAse (ribonuclease) as a parent domain, because of the behavioral and structural unpredictability inherent in the synthesis of any new protein, this suggestion does not render obvious every fusion protein in which a member of the RNAse species comprises the parent domain.

Page 3, Column 1, Paragraph 1 of **Doi et al** (cited by the Examiner in support of his assertion that **Doi et al** suggest the use of RNAse for designing stable bifunctional proteins) contains the following statement:

> "[C]onstruction of an insertional fusion protein is rather complicated, because it requires precise information on the parent domain structure to identify a suitable insertion site. Inappropriate design often leads to destabilization and inactivation of the insertional fusion protein."

Doi et al, the Examiner's Primary Reference, could not make the unpredictability of the stability of the structure of any contemplated fusion protein any clearer, and underscores why the fusion protein claimed in Claim 1 is not obvious. Where, as here, a fusion protein is claimed as a composition of matter, its stability, and hence its very existence, is not made obvious merely because its synthesis entailed the use of an RNAse as a parent (target) domain

In view of the foregoing responses to each and every point of reliance by the Examiner on his Primary Reference **Doi et al**, Applicants respectfully contend that Claims 1 & 2 and are not unpatentable over **Doi et al** standing alone, because **Doi et al** do not teach or suggest each and every feature or element of Claims 1 & 2, in particular, a ubiquitin-barnase fusion protein, claimed as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein".

The Examiner's Argument in Reliance upon Varshavsky

The Examiner asserted that **Varshavsky** teaches that:

- with cancer, cytotoxic therapies generally fail due to
  - their lack of selectivity [p.2094, Col. 1, ¶ 2] and
  - their inability to adjust to intracellular protein levels; and,
- conditionally specific cytotoxic regimes must possess
  - a multi-target combinatorial (positive/negative) selectivity [P. 2095, Col. 1. ¶4 and ¶5]; and,
  - codominance interference [P. 2095, Col. 2]wherein two signals in the same molecule function without interference in a mutually exclusive fashion to increase or decrease toxicity of the complex [Fig. 2] and [Fig. 4]

[DETAILED ACTION; PAGE 6 LAST FULL PARAGRAPH TO PAGE 7 FIRST PARTIAL PARAGRAPH]

This teaching is not cited by the Examiner as teaching or suggesting any feature or any element of any claim herein. Rather, the Examiner cites **Varshavsky** as

- teaching a ***method*** for synthesizing the ubiquitin-barnase fusion protein of Claims 1 and 2; and,
- providing a ***hypothetical motivation to*** synthesize the ubiquitin-barnase fusion protein of Claims 1 and 2, because of the performance defects of the anti-cancer cytotoxic therapies discussed by **Varshavsky**.

However, a closer reading of Varshavsky fails to reveal the teaching of any method for the synthesis of ubiquitin-barnase fusion protein. Significantly,

Varshavsky does not even contain the words "barnase," "ubiquitin," fusion protein or "RNAase" at all.

Rather, **Varshavsky** teaches that:

- "[A] conditionally cytotoxic therapeutic regimen that is exquisitely specific for a given cancer, and therefore would eliminate it without significant side effects, must possess, in most cases, a multi-target, combinatorial (positive/negative) selectivity"; and,
- "The challenge ... is to attain a multi-target, combinatorial selectivity in the setting of small drugs, where the immunogenicity and delivery problems are less severe. A solution ... invokes a modification of the earlier idea of co-dominant interference in conjunction with the new concept of anti-effectors."

Clearly, these teachings do not teach or suggest a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein" as in Claims 1 and 2

Moreover, **Varshavsky** does not provide any ***motivation*** for synthesizing a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein" as in Claims 1 and 2, because Claims 1

and 2:

- do not claim ***multi-target combinatorial selectivity*** as suggested by **Varshavsky** or a substantial equivalent thereof; and,
- do not claim ***co-dominant interference*** using ***anti-effectors***, as suggested by **Varshavsky** or a substantial equivalent thereof

The Examiner's Argument in Reliance upon Sevcik et al

Referring specifically to P. 47325, Col. 2 of their paper, the Examiner contended that **Sevcik et al** teach

- the cytotoxic ribonuclease protein *barnase* in complex with barstar for use in treating cancer;

and, referring specifically to p. 47329, Col. 1, ¶ 5 of their paper, the Examiner contended that **Sevcik et al**

- provide evidence that the conformational stability of barnase correlates with toxicity.

These teachings are not cited by the Examiner as teaching or suggesting any feature or any element of any claim herein. Rather, **Sevcik et al** are cited by the Examiner as allegedly teaching a method for creating a fusion protein using barnase, the creation being hypothetically driven by the motivation taught by **Varshavsky**.

As indicated in the foregoing section, **Varshavsky** does ***not*** provide any motivation for synthesizing a ubiquitin-barnase fusion protein. Moreover, a closer reading of the Examiner's citations within **Sevcik et al** reveals that **Sevcik**

et al teaches that:

- Barnase is the best characterized member of the prokaryotic subfamily of microbial ribonucleases; and,
- Barstar inhibits the enzymatic activity of Sa ribonucleases as it does barnase; and,
- Barnase may exist in a complex with barstar (the intracellular protein inhibitor of barnase that is co-expressed with barnase by *Bacillus amyloliquefaciens*)

Sevcik et al does ***not*** contain the term "fusion protein" and it does ***not*** teach or suggest a ***method*** for creating a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein" as in Claim 1.

The Examiner's Argument in Reliance upon Hochstrasser

The Examiner contends that **Hochstrasser** teaches that:

- ubiquitin (and ubiquitin-related proteins) as the prototypical example of a regulatory stratagem for covalently modifying another protein, thereby altering its [another protein's] physiological properties [p. E153, Col. 1, ¶ 1 and 2], because ubiquitin advantageously possesses a larger and more chemically varied surface area [p. El 53, Col. 1, ¶ 2].
- ubiquitin attached to activating and conjugating enzymes (i.e. ubiquitination) [Fig.2]; and,

- attached ubiquitin modules can function as reversible connectors [Fig.2]; and,
- attached ubiquitin modules could also link upstream synthetase to proteins or other molecules that regulate activity or localization of the enzyme [P. E156, Col. 1, ¶ 1].

However, these teachings do not teach or suggest a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein".

Rather, the Examiner has cited **Hochstrasser** as allegedly teaching a method for creating a fusion protein using ubiquitin, which creation is again hypothetically driven by the motivation allegedly taught by **Varshavsky**. And, as indicated in the foregoing section, **Varshavsky** does ***not*** provide any motivation for synthesizing a ubiquitin-barnase fusion protein.

A closer reading of **Hochstrasser** reveals that the word "barnase" does not appear in **Hochstrasser** and that the term "fusion protein" does not appear in **Hochstrasser**, confirming the Examiner's assertion that ubiquitin is the prototypical example of an intracellular polypeptide that ***covalently*** modifies another protein. Accordingly, while Hochstrasser teaches that ubiquitin functions by covalently modifying other proteins, it teaches or suggests nothing about creating ubiquitin-barnase fusion proteins, whether driven by the

hypothetical motivation attributed to **Varshavsky** or otherwise

The Examiner's Rejection of Claims 1 and 2

The Examiner rejected Claims 1 and 2 under 35 USC §103(a) as being made obvious by **Doi** et **al** in view of **Sevcik** et **al, Hochstrass**er and **Varshavsky.**

In view of the foregoing responses to each and every point of reliance by the Examiner on his Secondary References **Varshavsky, Sevcik** et **al**, and **Hochstrass**er, whether standing alone, in any combination among themselves, and in any combination with Primary Reference **Doi et al**, Applicants respectfully contend that Claims 1 & 2 and are not unpatentable over **Doi et al** in view of **Varshavsky, Sevcik et al**, and **Hochstrasser**

Alone or in combination, none of these references teach or suggest each and every feature or element of Claims 1 & 2, in particular, a ubiquitin-barnase fusion protein, claimed as a composition of matter, the structure of which is limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein".

Since the References marshaled by the Examiner do not expressly or impliedly suggest the claimed invention, the Examiner must present a convincing line of reasoning as to why one skilled in the art would have found the claimed invention to have been obvious in light of the teachings of the references.

The Examiner's line of reasoning is not convincing, because **Doi et al** teach insert domains whose amino acid lengths are ***less than*** the amino acid lengths of parent (target) domains - - in effect, the opposite of what is claimed in Claim 1 and Claim 2. Consequently, **Doi et al** necessarily fail to support a convincing line of reasoning as to why one skilled in the art would have found the claimed invention to have been obvious, alone or in light of the teachings of the Secondary References. Even if, ***arguendo***, **Doi et al** contained an express or implied motivation to combine the Examiner's Secondary References (which it does not), their combination would teach the creation of a fusion protein whose constituent insert domain would have ***fewer*** amino acids than its constituent parent (target) domain, an invention that is patentably distinct from that claimed in Claim 1 and Claim 2.

In view of Applicants' foregoing responses to each and every point of reliance by the Examiner on his Secondary References **Varshavsky**, **Sevcik et al**, and **Hochstrasser**, whether standing alone, in any combination among themselves, and in any combination with Primary Reference **Doi et al**, Applicants respectfully contend that the Examiner has not shown a proper motivation in these references or in the knowledge of one skilled in art to modify either the references or to combine their teachings.

The mere fact that references can be combined or modified does not render the resultant combination obvious, unless the prior art also suggests the desirability of the combination. Moreover, because **Doi et al** teach insert domains whose amino acid lengths are ***less than*** the amino acid lengths of parent (target)

domains - - in effect, the opposite of what is claimed in Claim 1 and Claim 2 - - **Doi et al** not only teach away from Claim 1 and Claim 2, but motivates a person skilled in the art to practice an invention that is, of necessity, patentably distinct from the invention claimed in Claim 1 and Claim 2.

The Examiner's Rejection of Claim 6

The Examiner acknowledged that **Doi et al**, **Sevcik et al**, **Hochstrasser**, and **Varshavsky** do not specifically teach limitations of claims 3, 4, and 5, but was silent on the teachings of **Sevcik et al**, **Hochstrasser**, and **Varshavsky** with regard to claim 6

[DETAILED ACTION: PAGE 8; SECOND FULL PARAGRAPH]

In light of Applicants' cancellation of Claim 6 herein, this omission is rendered moot.

However, the Examiner asserted that **Falnes et al** teach

- the inactivity of surface-bound toxins by exposure to low pH [p.615, Col. 2, 12]; as well as,
- *in vivo* degradation signals used to reduce the toxicity of a protein toxin [p.620, Col. 1, ¶ 2] and [p.621, Col. 1, ¶ 2],

which allegedly suggests disenabling proteins, as in claim 6.

[DETAILED ACTION: PAGE 8; SECOND FULL PARAGRAPH]

Again, in light of Applicants' cancelation of Claim 6 herein, this rejection is rendered moot.

The Examiner's Rejection of Claims 3-5

The Examiner acknowledged that **Doi et al**, **Sevcik et al**, **Hochstrasser**, and **Varshavsky** do not specifically teach limitations of claims 3, 4, and 5. [DETAILED ACTION: PAGE 8; SECOND FULL PARAGRAPH]

The Examiner's Argument in Reliance upon Pace et al, Wintrode et al and Falnes et al

However, as above, the Examiner asserted that **Falnes et al** teach

- the inactivity of surface-bound toxins by exposure to low pH [p.615, Col. 2, 12]; as well as,
- *in vivo* degradation signals used to reduce the toxicity of a protein toxin [p.620, Col. 1, ¶ 2] and [p.621, Col. 1, ¶ 2],

which allegedly suggests controllable effector signals as in claims 3-5. [DETAILED ACTION: PAGE 8;SECOND FULL PARAGRAPH]

The Examiner also asserted that **Pace et al** teach:

- urea denaturation of barnase [is] based on pH dependence [Abstract];
- barnase stability is controllable by pH;
- barnase has a maximum conformational stability at higher pH values (i.e. 5-6) [p. 2733, Col. 2, ¶ 5]; and,
- barnase accessibility in both folded and unfolded conformations [Table II],

as in claims 3-5.

However, the teachings of **Pace et al** are explicitly limited to the effect of ***urea*** as a denaturant on barnase structure and stability, as a function of pH, where barnase ***alone*** is presented in a pH-dependent solution of urea, rather than being a component of a fusion protein. **Pace et al** ***actually*** teach that:

- ***urea*** denaturation of barnase [is] based on pH dependence;
- barnase stability is controllable ***by urea as a function of pH***;
- barnase has a maximum conformational stability ***in the presence of urea*** at higher pH values;
- the ***Tyr*** and ***Trp*** residues in barnase in both folded and unfolded conformations are accessible in 8 mM ***urea***.

Consequently, **Pace et al** teach or suggest nothing about the folding or unfolding of barnase as a target domain of a fusion protein under the influence of pH, as in Claims 3-5

The Examiner further asserted that **Wintrode et al** teach:

- the thermodynamics of ubiquitin folding [Abstract];
- that ubiquitin is controllable by pH and is more stable at lower pH values [p.247, Col. 1, Results and Discussion];
- denaturation (i.e. folding) of ubiquitin is reversible and temperature dependent [p.247, Col. 2], and the folding/unfolding associated with energy quantities [Table IV],

as in claims 3-5.

However, the teachings of **Wintrode et al** are explicitly limited to the effect of pH on ubiquitin structure and stability, where ubiquitin ***alone*** is presented to

pH- and temperature-dependent solutions of non-denaturing glycine/HCl, or sodium acetate/acetic acid buffer systems, rather than being a component of a fusion protein. Subject to these ***patently distinctive*** limitations, **Pace et al** teach the points made out by the Examiner, but suggest nothing about the folding or unfolding of ubiquitin as an insert domain of a fusion protein under the influence of pH or temperature, as in claims 3-5.

Notwithstanding the absence of the foregoing suggestions, the Examiner concluded that it would have been obvious to someone of ordinary skill in the art at the time of the instant invention to create a mutually exclusive fusion protein using the methods of

1) **Doi et al** and
2) **Varshavsky**
3) **Hochstrasser** and additionally using pH to control the cooperative folding of thebarnase and ubiquitin, as taught by
4) **Wintrode et al** and
5) **Pace et al,**

as in claims 2-6, wherein the hypothetical motivation would have been to determine optimal pH and temperature values for controlling barnase-ubiquitin fusion protein toxicity, resulting in the practice of the instant claimed invention. One of skill in the art would have had a reasonable expectation of successfully combining the above teachings as both Pace et al. and Wintrode et al. teach effects of pH on protein folding.
[DETAILED ACTION: PAGE 9; LAST FULL PARAGRAPH TO PAGE 10; FIRST PARTIAL PARAGRAPH]

Quite aside from the:

- teaching away,
- absence of teaching, and
- absence of suggestion,

discussed above for **Doi et al** (in light of) **Varshavsky**, **Hochstrasser** and **Sevcik et al** proffered by the Examiner to reject Claims 1 and 2, according to the Examiner, **Falnes et al** , **Pace et al** and **Wintrode et al** must now be added to "practice" a "mutually exclusive fusion protein," using pH to control the cooperative folding of the barnase and ubiquitin.

While there is, in principle, no limitation on the number of references that may be combined to make an invention obvious, it strains both common sense and credulity to imagine that one of skill in the art would have found it obvious to create a ubiquitin-barnase fusion protein, limited by the explicit design constraint that "an amino-carboxyl length of the ... insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the ... target protein," by combining seven references, with publication dates spanning over 10 years, based upon the Examiner's proposed motivation to determine optimal pH and temperature values for controlling barnase-ubiquitin fusion protein toxicity.

Response Summary

Applicant has shown that any individual, combination, or sub-combination of the Examiner's references do ***not*** teach Applicant's ***whole invention***. In particular, any individual, combination, or sub-combination of the Examiner's

references does not teach a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that an amino-carboxyl length of the insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the target protein as claimed in the present application.

There is nothing in any of these references that would suggest or motivate their combination or sub-combination to invent a ubiquitin-barnase fusion protein, as a composition of matter, the structure of which is limited by the explicit design constraint that an amino-carboxyl length of the insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the target protein as claimed in the present application.

Provisional Obviousness-Type Double Patenting Rejection

A terminal disclaimer is being filed together with this response to overcome the Examiner's provisional rejection of Claims 1-6 and 8-11, under the judicially created doctrine of obviousness-type double patenting, as being unpatentable over claims 1-11 of co-pending Application No. 11/670,966.

Request for an Extension of Time

It is requested that a six-month extension of time be granted for the filing of this response. If any fees, including extension of time fees, are due as a result of this response, please charge Deposit Account No. 503033.

Remarks Regarding Change of Address:

The Office Action was sent to applicants' attorneys at his former address:

c/o Whiteman Osterman & Hanna LLP
One Commerce Plaza
Albany, New York 12260

Applicants respectfully request that all future correspondence for this patent application be sent to:

Sander Rabin MD JD
A Professional Corporation
Saratoga Technology Accelerator
125 High Rock Avenue
Saratoga Springs, New York 12866
VOICE: 518 527-8785
FAX: 518 935-9038
srabin1@nycap.rr.com

CONCLUSION

In summary, based on the preceding arguments, Applicants respectfully believe that all independent claims and dependent claims meet the acceptance criteria for allowance and therefore request favorable action. If the Examiner believes that anything further would be helpful to place the application in better condition for allowance, Applicants invite the Examiner to contact Applicants' representative at the telephone number listed below.

Respectfully submitted,
/Sander Rabin/
Sander Rabin, MD JD   November 05, 2007
A Professional Corporation
Registration No. 53,498
Customer No. 41672
Saratoga Technology Accelerator
125 High Rock Avenue
Saratoga Springs, New York 12866
VOICE: 518 527-8785
FAX: 518 935-9038
srabin1@nycap.rr.com

APPENDIX A:
"Clean" Version of Claim Listing

1. (Presently Amended) A fusion protein comprising a ubiquitin insert protein having an insert regulatory domain lying between an amino terminal and a carboxyl terminal of the ubiquitin insert protein; and, a barnase target protein having a surface loop that begins at an alpha carbon of an initial amino acid of the surface loop and terminates at an alpha carbon of a terminal amino acid of the surface loop, the surface loop comprising a cytotoxic target domain of the barnase target protein, wherein, the ubiquitin insert protein is inserted at a point within the surface loop between the alpha carbon of the initial amino acid of the surface loop and the alpha carbon of the terminal amino acid of the surface loop, such that an amino-carboxyl length of the ubiquitin insert protein is at least two-times greater than an alpha-carbon-alpha-carbon length of the barnase target protein.

2 (Previously Amended) The fusion protein of claim 1, wherein the insert regulatory domain exists in either a folded or unfolded conformation and the target cytotoxic domain exists in either a folded or unfolded conformation, the insert regulatory domain and the target cytotoxic domain comprising a cooperative and reversible conformational equilibrium such that if the insert regulatory domain is in its folded conformation, the target cytotoxic domain is in its unfolded conformation and vice versa.

3. (Currently Amended: CLEAN) The fusion protein of claim 2, wherein the target cytotoxic domain folds under the influence of a first controllable effector signal and the insert regulatory domain folds under the influence of a second controllable effector signal.

4. (Previously Amended) The fusion protein of claim 3, wherein the first controllable effector signal is selected from the group comprising ligand binding, pH, temperature, chemical denaturants, or mutations in either the insert domain or the target domain.

5. (Previously Amended) The fusion protein of claim 3, wherein the second controllable effector signal is selected from the group comprising ligand binding, pH, temperature, chemical denaturants, or mutations in either the insert domain or the target domain.

6. (Currently Cancelled) The fusion protein of claim 2, wherein the insert domain and the target domain are disenabled from simultaneously co-existing in their respective folded conformations.

7. (Previously Cancelled) The fusion protein of claim 2, wherein said insert domain and said target domain are disenabled from simultaneously co-existing in their respective unfolded conformations.

8. (Currently Cancelled) The fusion protein of claim 2, wherein any excess of the first quantity of free energy of the insert regulatory domain that is not

necessary to stabilize the insert regulatory domain in its folded conformation is spontaneously transferred, through the structure of the fusion protein, to the target cytotoxic domain to unfold it from its folded conformation.

9. (Currently Cancelled) The fusion protein of claim 2, wherein any excess of the second quantity of free energy of the target cytotoxic domain that is not necessary to stabilize the target cytotoxic domain in its folded conformation is spontaneously transferred, through the structure of the fusion protein, to the insert regulatory domain to unfold it from its folded conformation.

10. (Previously Amended) The fusion protein of claim 2, wherein the insert protein comprises human ubiquitin, the insert regulatory domain comprises a regulatory domain of human ubiquitin, the target protein comprises barnase, the target cytotoxic domain comprises a cytotoxic domain of barnase, the amino-carboxyl length is about 38 Å, the initial amino acid of the surface loop comprises proline in the number 64 position ("Pro64"), the terminal amino acid of the surface loop comprises threonine in the number 70 position ("Thr70"), and the alpha-carbon-alpha-carbon length is about 10.4 Å.

11. (Currently Amended: CLEAN) The fusion protein of claim 10 wherein the regulatory domain of human ubiquitin and the cytotoxic domain of barnase comprise a cooperative and reversible conformational equilibrium, which conformational equilibrium is subject to the influence of the controllable first and second effector signals.

12. (Previously Cancelled) A method for the production of a protein comprising the steps of:

a. selecting a linker containing first and second restriction sites between a Lys66 and a Ser67 codon of a barnase gene;

b. using said first and second restriction sites of said linker to operationally insert a ubiquitin gene between two amino-acid codons of said linker, thereby creating a ubiquitin-barnase fusion gene;

c. fully sequencing said ubiquitin-barnase fusion gene to verify its integrity;

d. using enzymes to operationally insert said ubiquitin-barnase fusion gene into any plasmid of a BL21 (DE3) family, thereby creating an interim ubiquitin-barnase fusion expression plasmid;

e. operationally inserting a gene for barstar and its natural promoter from *Bacillus amyloliquifaciens* into said interim ubiquitin-barnase fusion expression plasmid, thereby creating a ubiquitin-barnase fusion-barstar complex plasmid;

f. cloning said gene for barstar into a T7 promoter-containing plasmid conferring resistance to an antibiotic other than ampicillin onto cells transformed by said T7 promoter-containing plasmid, thereby creating a barstar plasmid;

g. transforming *E. coli BL21* (DE3) cells grown at about 20 to 37 degrees C in any medium compatible with E. coli growth using both said barstar plasmid and said ubiquitin-barnase fusion-barstar complex plasmid, and inducing said *E. coli* BL21 (DE3) cells with about 100 mg/L isopropyl b-D-thiogalactopyranoside;

h. harvesting said transformed *E. coli* cells by centrifugation after about 2 to 12

hours; after said induction;

i. placing said harvested *E. coli* cells in10 mM sodium phosphate at a pH of 7.5, thereby creating a solution of harvested *E. coli* cells;

j. lysing said solution of harvested *E. coli* cells by repeated freeze-thaw cycles in the presence of about 10mg/liter lysozyme, thereby creating a lysate;

k. adding about 10 mg/liter DNase I to reduce the viscosity of said lysate;

l. centrifuging said reduced viscosity lysate to remove insolubles, thereby forming a supernatant;

m. adding about 8 M urea to said supernatant to dissociate bound barstar;

n. Removing said dissociated barstar from said supernatant by passing said supernatant through an anion exchange chromatography resin to yield a solution;

o. loading said solution onto a cation exchange column;

p. washing said solution with about 10 mM sodium phosphate (pH about 7.5) and about 6 M urea;

q. eluting said solution using a 0 to 0.2 M NaCl gradient;

r. Removing said urea from said dilution by dialysis against double-distilled water to yield barnase-ubiquitin fusion protein.